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Review Paper

Overview of the detection methods for equilibrium dissociation constant K_D of drug-receptor interaction

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ABSTRACT

Drug-receptor interaction plays an important role in a series of biological effects, such as cell proliferation, immune response, tumor metastasis, and drug delivery. Therefore, the research on drug-receptor interaction is growing rapidly. The equilibrium dissociation constant (K_D) is the basic parameter to evaluate the binding property of the drug-receptor. Thus, a variety of analytical methods have been established to determine the K_D values, including radioligand binding assay, surface plasmon resonance method, fluorescence energy resonance transfer method, affinity chromatography, and isothermal titration calorimetry. With the invention and innovation of new technology and analysis method, there is a deep exploration and comprehension about drug-receptor interaction. This review discusses the different methods of determining the K_D values, and analyzes the applicability and the characteristic of each analytical method. Conclusively, the aim is to provide the guidance for researchers to utilize the most appropriate analytical tool to determine the K_D values.

Contents

1. Introduction

The type of drug target is divided into receptor, enzyme, nucleic acid, and so on. There are about 40% drugs which interact with the corresponding receptors in order to exert their pharmacological effects. When the ligands (first messenger) combine with the corresponding receptor, a signal cascade reaction occurs through the second messenger in the cell, resulting in a series of biological effects, such as immune response and cell proliferation [1–3]. Therefore, it is very necessary to study the interaction between drugs and receptors, which contributes to understanding the mechanisms of drugs [4–8]. The equilibrium dissociation constant (K_D) is the basic parameter to evaluate the binding properties of the drug-receptor [9–11]. Thus, it is of great importance to determine the K_D values of the drugs.

A variety of analytical methods have been established to determine the K_D values since the 1960s, including radioligand binding assay (RBA) [12], surface plasmon resonance (SPR) [13], fluorescence energy resonance transfer method (FRET) [14], affinity chromatography [15], and isothermal titration calorimetry (ITC) [16].

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The main purpose of this review is to analyze the applicability and characteristic of each analytical method in order to provide the guidance for researchers to choose an appropriate analytical tool to study the ligand-receptor interaction.

2. Radioligand binding assay

In the early 1960s, radiolabelled nuclide was used in the receptor pharmacology study. Based on occupation theory [17], RBA method was established. RBA is based on the interaction between radiolabeled ligand and receptor. RBA can be used to study the interaction between receptor and hormones, neurotransmitters, growth factors and drugs, as well as the interaction between the receptor and the second messenger [18].

As shown in Table 1, RBA can be used to determine the affinity constant, dissociation constant and the number of binding receptors [19–34]. The key condition of receptor binding experiments is to prepare an excellent radioligand. The basic requirements of radioligand are high radioactivity, high affinity, high specificity and stability [22,35,36]. The ability to prepare novel and selective radioligands facilitates the study of drug-receptor interaction, and RBA provides an effective tool for studying the mechanisms of drugs at molecular level [37,38]. A variety of receptor materials can be used in the RBA method, such as the cell membrane obtained from cell and tissue [19–28], intact cells [29,30], tissue slice [31–34], and engineered protein samples [38,39].

RBA method provides a sensitive detection method for determining K_D values and promotes the study of receptor pharmacology [37,38]. However, it is not very easy to synthesize the specific radioligands which are the essential elements for RBA method, and radioactive contamination should be prevented during the experiment. So the application of RBA method is greatly limited.

3. Surface plasmon resonance technique

SPR technique, which has been rapidly developed in recent years, is a sensitive and specific technique for the analysis of biomolecular interactions [40]. SPR is based on the principle that the incident light can resonate with the plasma on the metal surface during the total reflection. SPR is utilized to detect whether biological molecules interact with each other, and further explore the specificity of the interaction, kinetic parameters and affinity of the interaction [41–43]. SPR technique provides a powerful and nondestructive tool for cell sorting, cell surface characterization, protein-protein interaction, protein-small molecule interaction, and drug discovery [41,43–45].

SPR is a label-free and real-time detection method for monitoring biomolecular interactions [40]. In recent years, SPR has become a rapid developmental technology for studying the interaction between membrane protein receptors and ligands [44–56], which is shown in Table 2. Because of the high-throughput screening characteristic, SPR has been widely used in the identification of drug targets and the optimization of lead compounds [44–48].

4. Affinity chromatography

In the affinity chromatography, biological macromolecules bind in the carrier surface through chemical reaction. Affinity chromatography utilizes the liquid chromatography method to study the interaction between drugs and biological macromolecules [57–59]. As shown in Table 3, affinity chromatography is widely used in biochemistry, molecular biology, and genomics. It is becoming a commonly used method in the interaction of drug and biological macromolecules [60,61].

The frontal analysis and zonal elution method are utilized to determine the K_D values. Wainer and Hage group have done a lot of work to characterize the affinity of drugs with biomolecules [62–65]. The frontal analysis is mainly conducted by adding the analytes into the mobile phase without injection. Each drug solution with different concentrations is continuously applied to the column until a breakthrough curve with a level plateau is produced. The K_D values can be determined by analyzing the series of breakthrough curves [62,66–68]. Zonal elution method is performed by using a site-specific maker in the mobile phase and injecting the analytes. The K_D values of the analytes at a specific site are calculated by investigating the capacity factor of the analytes with the increasing concentration of the marker in the mobile phase [69,70].

Cell membrane chromatography (CMC) is a kind of bionic affinity chromatography, in which the membrane receptors are prepared as cell membrane stationary phase (CMSP), and is used in determining the interaction between drug and membrane receptors [71–74]. With the development of molecular biology, it is

Table 1
The RBA method and application examples.

No.	Receptor	Drug	Receptor material	K_D values	References
1	Glycine transporter-1	CHIBA-3007	Rat brain membranes	1.61 (± 0.16) × 10^{-9} M	[19]
2	α7 nicotinic acetylcholine receptors	CHIBA-1006	Rat brain membranes	88.2 (± 21.4) × 10^{-9} M	[20]
3	Opioid receptor	TICP[psi]	Rat brain membranes	0.35 × 10^{-9} M	[21]
4	5-HT(1A) receptors	WAY100635	Rat hippocampal membranes	87 (± 4) × 10^{-12} M	[22]
5	Dopamine D1 receptor	SCH23390	Sheep brain striatum membranes	56 (± 8) × 10^{-9} M	[23]
6	Histamine H3 receptor	Thioperamide	Rat cerebral cortex	0.80 (± 0.06) × 10^{-9} M	[24]
7	Adenosine A2A receptors	ZM241385	Rat striatum membranes Transfected CHO cell membranes	0.14 × 10^{-9} M 0.23 × 10^{-9} M	[25]
8	Platelet-activating factor (PAF) receptor	L-659,989	Rabbit platelet membranes	1.60 (± 0.20) × 10^{-9} M	[26]
9	Bradykinin B2 receptors	PIP HOE 140	Guinea pig ileal membranes	15 × 10^{-12} M	[27]
10	Thromboxane (TP-) receptor	GR32191	Human platelets membranes Human platelets	2.1 × 10^{-9} M 2.2 × 10^{-9} M	[28]
11	Human angiotensin II AT1 receptor	Olmesartan Telmisartan	CHO-hAT(1) cells	0.091 × 10^{-9} M 0.12 × 10^{-9} M	[29]
12	Glucocorticoid receptor	Dexamethasone	Peripheral blood mononuclear cells	66.194 × 10^{-9} M	[30]
13	Histamine H1-receptors	Mepyramine	Bovine retinal blood vessels	2.78 (± 0.32) × 10^{-9} M	[31]
14	Calcium channels	Nitrendipine	Rat brain synaptosomes	0.35 × 10^{-9} M	[32]
15	NK1 receptor	CP96,345	Guinea pig lung	0.12 (± 0.03) × 10^{-9} M	[33]
16	vasopressin V2-receptors	DDAVP	Rat kidney	0.76 × 10^{-9} M	[34]

Table 2
The SPR method and application examples.

No.	Receptor	Drug	Receptor material	K_D values	References
1	EGFR	EGF	EGFR protein	0.177×10^{-6} M	[44]
		GE11		0.459×10^{-3} M	
		mAb LA1		2.07×10^{-9} M	
2	Subendothelial collagens	vWf	Purified protein	$2.03(\pm 0.04) \times 10^{-9}$ M	[45]
3	Pr55(Gag)	1,4,5-IP3	Purified protein	2170×10^{-6} M	[46]
		di-C(8)-PI		186×10^{-6} M	
		di-C(8)-PI(4,5)P2		47.4×10^{-6} M	
4	VEGFR2 D3	Nanobody against NTV(1–4)	HUVEC cell	$49(\pm 1.8) \times 10^{-9}$ M	[47]
5	CD56	Monoclonal antibodies m900	Cancer cell	2.9×10^{-9} M	[48]
		Monoclonal antibodies m906		4.5×10^{-9} M	
6	Grp1 PH domain	Biotinylated Ins(1,3,4,5)P4	Rat brain membranes	0.14×10^{-6} M	[49]
7	Angiotensin converting enzyme	Lisinopril	Angiotensin converting enzyme	1.78×10^{-9} M	[50]
8	rKDR1–3	VEGF165	rKDR1–3 protein	57.4×10^{-9} M	[51]
9	Lipoprotein lipase	Bis-ANS	Purified protein	$(0.10 - 0.26) \times 10^{-6}$ M	[52]
10	CL-43	Yeast mannan	Purified protein	$(2.68 - 2.72) \times 10^{-8}$ M	[53]
11	Human glycophorin A	Nanobody IH4	Human glycophorin A	33×10^{-9} M	[54]
12	Cyclophilin A	Trp-Gly-Pro	Cyclophilin A	3.41×10^{-6} M	[55]
13	Collagen glycoprotein VI	GABA	Human platelets	41.4×10^{-9} M	[56]

Table 3
The affinity chromatography method and application examples.

No.	Receptor	Drug	Receptor material	K_D values	References
1	Estrogen receptor	Diethylstilbestrol	Purified protein	ERRγ, 237×10^{-9} M	[62]
				ERRα, 929×10^{-9} M	
2	α3β4nicotinic acetylcholine receptor	Dextromethorphan	α3β4-nAChR cell membrane	K_a:$23.40(\pm 0.36) \times 10^6$ M^{-1}	[63]
		Levomethorphan		K_a:$12.01(\pm 0.23) \times 10^6$ M^{-1}	
3	Cannabinoid receptor CB1	Win-55, MA, ACEA	KU-812 cell membrane	$(8.6 \pm 8.3) \times 10^{-9}$ M, $(19.8 \pm 10.9) \times 10^{-9}$ M, $(0.74 \pm 0.22) \times 10^{-9}$ M	[64]
	Cannabinoid receptor CB2			$(0.37 \pm 0.12) \times 10^{-9}$ M, $(653 \pm 182) \times 10^{-9}$ M, $(5200 \pm 2.5) \times 10^{-9}$ M	
4	PKCα	Chelerythrine	Purified protein	698×10^{-9} M	[66]
5	μ opioid receptors	Naloxone	CHO-μ opioid receptors cell membrane	110×10^{-9} M	[67]
	κ opioid receptors	U69593	CHO-κ opioid receptors cell membrane	84×10^{-9} M	
6	L-type calcium channel	Nifedipine	VSMC	$(3.36 \pm 0.28) \times 10^{-6}$ M	[72]
		Nimodipine		$(1.34 \pm 0.15) \times 10^{-6}$ M	
		Nitrendipine		$(6.83 \pm 0.48) \times 10^{-7}$ M	
		Nicardipine		$(1.23 \pm 0.16) \times 10^{-7}$ M	
		Amlodipine		$(1.09 \pm 0.09) \times 10^{-7}$ M	
		Verapamil		$(8.51 \pm 0.61) \times 10^{-8}$ M	
7	α_{1A} adrenoreceptor	Tamsulosin	α_{1A}/HEK293 cell membrane	$(1.87 \pm 0.13) \times 10^{-6}$ M	[74]
		5-methylurapidil		$(2.86 \pm 0.20) \times 10^{-6}$ M	
		Doxazosin		$(3.01 \pm 0.19) \times 10^{-6}$ M	
		Terazosin		$(3.44 \pm 0.19) \times 10^{-6}$ M	
		Alfuzosin		$(3.50 \pm 0.21) \times 10^{-6}$ M	
8	Histamine H_1 receptor	Azelastine	H_1R/HEK293 cell membrane	$(8.72 \pm 0.21) \times 10^{-7}$ M	[75]
		Cyproheptadine		$(9.12 \pm 0.26) \times 10^{-7}$ M	
		Doxipine		$(9.90 \pm 0.18) \times 10^{-7}$ M	
		Astemizole		$(1.42 \pm 0.13) \times 10^{-6}$ M	
		Chlorpheniramine		$(2.25 \pm 0.36) \times 10^{-6}$ M	
		Diphenhydramine		$(3.10 \pm 0.27) \times 10^{-6}$ M	

possible to construct cell lines with high expression of specific receptors, which makes the CMC method have stronger specificity and selectivity. Based on the development of CMC, a variety of CMC models such as L-type calcium channel, α_{1A} adrenergic receptor, histamine H_1 receptor and vascular endothelial growth factor receptor models are established in order to determine the K_D values [75–78].

Affinity chromatography plays a very important role in the study of drug-receptor interaction. The methods of binding receptor to the carrier surface are divided into chemical bonding and physical adsorption. However, after the immobilization of biological macromolecules via chemical bonding, their original configurations and even functions are largely "lost". While physical adsorption can largely retain the three-dimensional configurations and biological activities of membrane receptors and can accurately reveal the drug-membrane receptor interaction process in vivo.

5. Fluorescence energy resonance transfer method

Fluorescence spectroscopy is the most popular technique in the field of biology and medicine, which leads people to the microcosmic world of bio-medicine. The theoretical basis for FRET is a nonradiative energy transfer between two fluorescent molecules (D and A, whose excitation spectra are partially overlapped) that are located close to each other (less than 10 nm) [79–81]. FRET can

be used to study receptor-ligand interactions, affinity constants, receptor dimerization, and so on [82–84].

FRET has been widely used in drug-receptor affinity studies under equilibrium condition with no need to separate the free and combinative ligands [85–87]. Piehler group studied the interaction of IFNR2 with Ifnar1-H10 and measured its K_D value to be 5 μM by FRET technique [88]. Domanov et al. [89] also used the FRET technique to study the interaction between cytochrome c and bilayer phospholipid membranes and found a K_D value of 0.2–0.4 μM.

FRET has the following advantages compared with other methods. The first is high sensitivity, and it is now possible to study single receptor molecules in this way. Moreover, FRET can selectively study intermolecular interactions under physiological conditions (living cell states) [90,91]. Another advantage is that a variety of fluorescent probes can be obtained commercially. The fluorescent probes can be used to label molecules with no fluorescence properties, thus greatly broadening the research approach. Combined with its high spatial resolution, FRET becomes an excellent tool for studying receptor-ligand interactions [92,93].

6. Isothermal titration calorimetry

ITC is a technique based on the reaction heat to quantify the interactions of various biomolecules. As a kind of rapid and direct tool without markers, ITC can detect any heat changes of biochemical reaction process. ITC is widely used in molecular biology research, drug design and structure optimization, and drug mechanism studies [94,95].

Micro calorimeter with high sensitivity and high automation is used to monitor and record the calorimetric curve of the reaction process continuously and accurately. ITC, an in situ, on-line and non-destructive method, provides the thermodynamic and kinetic information (eg, binding constant (K_D), reaction stoichiometry (n), enthalpy (ΔH) and entropy (ΔS) [96,97]. ITC can be also used to study the properties of drug-receptor interaction by directly detecting the heat changes during the process of biochemical reaction [98,99].

By means of the ITC method, Li et al. [16] studied the interaction of neomycin and tobramycin with MLL protein, and found that the K_D values are18.8 for neomycin and 59.9 μM for tobramycin, respectively. Daddaoua group found that only 2-ketoglutarate could act on PtxS with an affinity constant K_D of 15 μM from glucose, ketoglucose and 2-ketoglucose by ITC technology [100].

Without any modification of receptors and ligands, ITC can directly determine the affinity of the drug-receptor under natural conditions [101]. ITC can not only determine the binding affinity, but also clarify the potential mechanism of molecular interactions. ITC is able to confirm the expected binding targets in the drug discovery process, deeply understand the structure-function relationship, and provide the guidance for candidate compounds selection and lead compounds optimization [102,103].

7. Conclusion

In all, the above five methods are all effective analytical tools to study the ligand-receptor interaction. RBA and FRET methods both provide high sensitivity, while both of them need the specific label (radiolabeled ligand for RBA, fluorescent label for FRET), which limits the application of the methods. Affinity chromatography is a nondestructive and dynamical method to study the ligand-receptor interaction, but the sensitivity is limited by the detector of HPLC. As SPR and ITC methods are highly sensitive and nondestructive, they provide powerful tools for studying drug-receptor interaction. Therefore, high sensitive and nondestructive analysis methods play a crucial role in the exploration of ligand-receptor interaction.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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